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CASE REPORT

Reconstitution of NK cells expressing KIR3DL1 is associated with reduced NK cell activity and relapse of CML after allogeneic hematopoietic stem cell transplantation

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Abstract

Although the prognosis of chronic myeloid leukemia (CML) in blastic crisis remains poor, some patients achieve long-term remission after allogeneic hematopoietic stem cell transplantation (allo-HSCT). This may be attributable to graft-versus-leukemia (GVL) effects by donor lymphocytes, but their regulating mechanisms are unclear. Antitumor natural killer (NK) cell immunity is assumed to be important in CML, and we have previously shown that allelic polymorphisms of killer immunoglobulin-like receptors (*KIRs*) and histocompatibility leukocyte antigens (*HLAs*) are associated with the response of CML to tyrosine kinase inhibitors. Here, we report a case of CML in blastic phase who received HLA-matched but KIR3DL1 allelic-mismatched allo-HSCT. After transplant, decreased BCR–ABL transcript levels and enhanced NK cell activity were transiently observed. However, reconstitution of KIR3DL1-expressing NK cells occurred, which was associated with diminished NK cell activity and increased BCR–ABL. This case indicates the potential significance of KIR3DL1 in NK cell-mediated GVL activity following allo-HSCT. To the best of our knowledge, this is the first report to analyze the association between sequential KIR3DL1 expression and activity of NK cells after allo-HSCT. Selecting donors with KIR3DL1-null alleles may maintain competent GVL effects and provide improved outcomes in allo-HSCT for CML.

Keywords Chronic myeloid leukemia (CML) · Allogeneic peripheral blood stem cell transplantation · Natural killer cell (NK cell) · Killer immunoglobulin-like receptor · KIR3DL1

Introduction

Although tyrosine kinase inhibitors (TKIs) afford long-term survival in patients with chronic myeloid leukemia (CML) in chronic phase, the prognosis of CML in blastic crisis (CML-BC) remains poor [1]. In those cases, myeloablative chemotherapy and allogeneic hematopoietic stem cell transplantation (allo-HSCT) can sometimes lead to long-term remission [2]. Given that CML-BC is resistant to cytotoxic chemotherapy, remission may derive from graft-versus-leukemia (GVL) effects by donor lymphocytes [3]. Whereas T cells play major roles in GVL, natural killer (NK) cell-associated GVL activity is also assumed to be important [4]. However, their regulating factors are unclear.

NK cells quickly reconstitute after allo-HSCT, and may be associated with potent GVL effects [4-6]. Given that NK cell activity and cytotoxicity are determined through the interactions of clonally distributed receptors called killer

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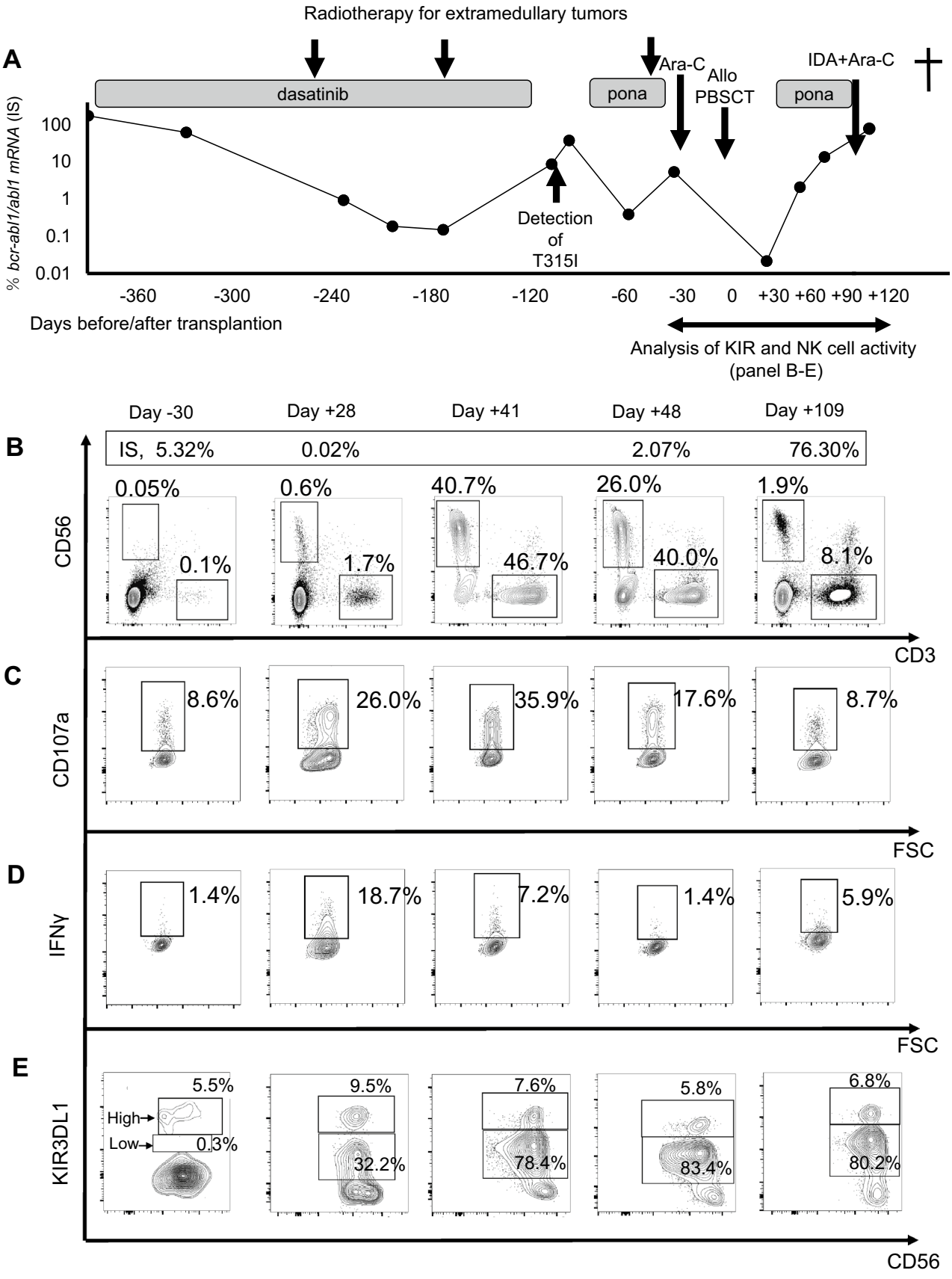
Radiotherapy for extramedullary tumors
A
dasatinib
pona
Ara-C
Allo PBSCT
pona
IDA+Ara-C
% bcr-abl1/abl1 mRNA (IS)
100
10
1
0.1
0.01
Detection of T315I
-360 -300 -240 -180 -120 -60 -30 0 +30 +60 +90 +120
Days before/after transplantion
Analysis of KIR and NK cell activity (panel B-E)
Day -30 Day +28 Day +41 Day +48 Day +109
B
IS, 5.32% 0.02% 2.07% 76.30%
0.05% 0.1%
0.6% 1.7%
40.7% 46.7%
26.0% 40.0%
1.9% 8.1%
CD56
CD3
C
8.6%
26.0%
35.9%
17.6%
8.7%
CD107a
FSC
D
1.4%
18.7%
7.2%
1.4%
5.9%
IFNγ
FSC
E
5.5%
High
Low
0.3%
9.5%
32.2%
7.6%
78.4%
5.8%
83.4%
6.8%
80.2%
KIR3DL1
CD56

◂**Fig. 1** Clinical course. **a** *BCR–ABL1/ABL1* transcript levels before and after allo-HSCT are shown. **b** Sequential analyses of NK cell ($CD3^-CD56^+$) frequencies within PBMCs are shown. CD107a degranulation (**c**) and IFN-γ production (**d**) of NK cells ($CD3^-CD16^+CD56^+$) are shown. **e** KIR3DL1 protein expression on NK cells is shown. *pona* ponatinib, *Ara-C* cytarabine, *allo-PBSCT* allogenic peripheral blood stem cell transplantation, *IDA + Ara-C* idarubicin plus cytarabine

immunoglobulin-like receptors (KIRs) with histocompatibility leukocyte antigens (HLAs) [7, 8], GVL may be associated with polymorphisms of these molecules in both donors and recipients. Although it is of note that KIR-expressing NK cells harvested after allo-HSCT are hyporesponsive to the human CML-BC cell line K562 ex vivo [9], its clinical significance is not clear. The lack of recipient HLAs specific to donor KIRs may be associated with potent GVL activity by donor NK cells [10]. However, the results of other similar analyses have been inconsistent [11-13], and the role of *KIR* polymorphisms in allo-HSCT remains controversial.

Recently, next-generation sequencing (NGS) revealed that *KIRs* have a high degree of allelic polymorphism, which is associated with their protein expression levels and functional diversity [14]. In particular, *KIR3DL1* has diverse allelic polymorphism, and KIR3DL1 protein expression levels of high, low, and null subtypes are associated with their alleles [15, 16]. The most common *KIR3DL1* allele in Japanese population is **015* allele (45.4%), which encodes high KIR3DL1 expression. Second common *KIR3DL1* allele is **007* (15.8%, low expression), third is *3DS1*013* (15.1%), fourth is **005* (9.9%, low), and fifth is **001* (5.9%, high), whereas $KIR3DL1^{null}$ allele is rare (0.7%) [17]. On the other hand, *HLA-B* alleles and some of *HLA-A* alleles can be divided into Bw4 or Bw6 subtypes depending on their serological epitopes. HLA-Bw4 is further subdivided into Bw4-80I and Bw4-80T subtypes depending on an amino acid dimorphism of isoleucine or threonine at position 80 [18]. For example, we classify HLA-A*24:02 into HLA-Bw4-80I, B*07:02 into Bw6, and B*52:01 into HLA-Bw4-80I according to this subdivision. Allelic combinations of *KIR3DL1* and *HLA-B* determine their interaction avidity; e.g., $KIR3DL1^{high}$ binds HLA-Bw4-80I more strongly than Bw4-80T [19, 20]. Interestingly, their combinations and associated binding avidity are associated with clinical prognosis in several different diseases [19, 21]. Actually, we have found that allelic polymorphisms of *KIR3DL1* and *HLA-B* may predict prognosis of TKI-treated CML [17]. Given that KIR3DL1 is an inhibitory KIR, no interaction between KIR3DL1 and HLA-B may be associated with potent NK cell activity, and conversely, their physical interaction may be associated with diminished NK cell immunity.

Here, we report a CML case with megakaryoblastic transformation who received *HLA*-matched allo-HSCT. Whereas the main population of NK cells were negative for KIR3DL1 protein before transplant, reconstitution of KIR3DL1-expressing NK cells was observed after transplant. However, it was associated with suppressed NK cell activity and increased tumor burden, which indicates the potential significance of KIRs in NK cell-mediated GVL effects after allo-HSCT.

Methods

Genotyping of *HLA* and *KIR3DL1*

HLA and *KIR3DL1* alleles were genotyped with DNA from the patient's peripheral blood mononuclear cells with written informed consent. Illumina MiSeq system with HLA version 3.0 and KIR version 1.0 IGS kits (Scisco Genetics Inc.) was used as previously described [17]. All the procedures in this study were carried out in accordance with the ethical standards of institutional (Saga University) and national research committees, and the Declaration of Helsinki (UMIN-CTR, ID: R000020356).

Flow cytometry

The following antibodies were used for staining: FITC-anti-CD3 (OKT3, TONBO Biosciences, San Diego, CA, USA), PE-Cy7-anti-CD56 (HCD56), Pacific Blue-anti-CD107a (H4A3, BioLegend, San Diego, CA, USA), APC-Cy7-anti-CD16 (3G8, BD Biosciences, San Jose, CA, USA), APC-anti-CD158a (REA284, Miltenyi Biotec, Ltd, Bisley, Surrey, United Kingdom), PE-anti-CD158e (DX9, BD Biosciences, San Jose, CA, USA), and V450-IFN- γ (B27, BD Biosciences, San Jose, CA, USA). Fixation and permeabilization buffer sets (Thermo Fisher Scientific, Waltham, MA, USA) were used for staining intracellular IFN-γ. Stained samples were analyzed using an FACSVerse cytometer (BD Biosciences, San Jose, CA, USA) and FlowJo software (Tree Star, Ashland, OR, USA).

Peripheral blood mononuclear cells were co-cultured with the CML-BC cell line K562, and NK cell activity was evaluated by determining expression of CD107a (which positively correlates with the level of degranulation) and intracellular IFN-γ among $CD3^-CD16^+CD56^+$ gated cells by flow cytometry [17].

Case presentation

A 35-year-old man was referred to our hospital due to leukocytosis discovered during a medical check-up. He was diagnosed with CML in accelerated phase with the Philadelphia chromosome and trisomy 8 abnormality, which was defined as a major route of additional chromosomal abnormality.

Treatment with the second-generation TKI dasatinib was initially effective, and the *BCR–ABL* transcript level quantified according to the International Scale (IS) rapidly decreased from 172.3 to 0.9% in 6 months. However, multiple extramedullary tumors soon developed, which required radiotherapy (Fig. 1a). At 10 months after the initiation of dasatinib, the *T315I BCR–ABL* kinase domain mutation was detected with rapidly increasing levels of *BCR–ABL* fusion transcript (Fig. 1a). Although ponatinib was transiently effective rendering the *T315I* mutation undetectable, an extramedullary tumor developed in the left shoulder 2 months later, and the *T315I* mutation reappeared. Pathological findings of the biopsied tissue from the left humerus showed abundant infiltration of monotonous blastic cells that were positive for CD34 and CD41 but negative for myeloperoxidase (Fig. 2). A diagnosis of megakaryoblastic transformed CML with resistance to ponatinib was made.

Following low-dose cytarabine therapy, the patient received allogeneic peripheral blood stem cell transplantation (allo-PBSCT) from an HLA-matched sibling donor. The patient and the donor had following HLA class I alleles: *A*24:02, *26:01; B*07:02, *52:01;* and *C*07:02, *12:02*, and *KIR3DL1* allele of the recipient was **015*. The patient received fludarabine, busulfan, and melphalan as conditioning, and cyclosporine and mycophenolate mofetil were administered as immunosuppressants. Neutrophil and platelet engraftment were achieved by day + 15 after transplant. Full-donor chimerism was confirmed and the level of *BCR–ABL* transcript was markedly decreased (0.02%) on day + 28 (Fig. 1a). However, CML relapsed with an increased *BCR–ABL* level at day + 48, and the patient died on day + 115 due to disease progression following no response to treatments.

Over the course of the disease, the frequencies of NK cells in peripheral blood, their KIR3DL1 expression, and activation status in response to K562 cells were sequentially analyzed. Whereas the number of NK cell ($CD3^-CD16^+CD56^+$) population was limited before transplant, they quickly engrafted by day + 41, after which they decreased (days + 48 and + 109; Fig. 1b). CD107a expression and IFN-γ production that reflect NK cell ($CD3^-CD16^+CD56^+$ cell) activity also peaked at days + 28 and + 41, and decreased thereafter. Notably, NK cell frequencies and activity were inversely associated with the IS levels of *BCR–ABL* after transplant (Fig. 1c, d). In addition, whereas the main population of NK cells was negative for KIR3DL1 before transplant, NK cells with KIR3DL1 expression gradually dominated after transplant. Given the difference in KIR3DL1 expression levels and complete chimera status, it can be attributed to reconstitution with donor NK cells. Chronologically, gradual reconstitution of $KIR3DL1^+$ cells coincided with decreased NK cell population (Fig. 1b) and their hypo-responsiveness (Fig. 1c, d), and eventually increased *BCR–ABL* IS levels (Fig. 1a). We think that the donor and the recipient had different *KIR3DL1* alleles (KIR allelic-mismatched transplant) in this case. On the other hand, KIR ligand-mismatched transplant is defined in transplants that the recipient does not have ligand HLA for donor KIRs. At this point, our transplant was not KIR ligand-mismatched transplant, as the donor and the recipient had a common ligand HLA-Bw4 for KIR3DL1.

Discussion

Here, we present the clinical course of a patient with megakaryoblastic transformed CML who received *HLA*-matched, *KIR3DL1* allelic-mismatched allo-PBSCT. Before transplant, tumor cells were resistant to chemotherapeutic agents, and lymphocytes including NK cells were scarce. After transplant, NK cells with enhanced activity transiently increased, which was associated with reduced *BCR–ABL* levels. However, reconstitution of KIR3DL1-expressing NK cells was associated with decreased NK cells and the increase in *BCR–ABL* levels, which implies that signaling through KIR3DL1 protein suppresses antitumor NK cell cytotoxicity.

KIR3DL1 is one of the most polymorphic *KIRs*, and its ligand *HLA-Bw4* also has an isoleucine and threonine dimorphism at position 80 [22]. Several reports suggest

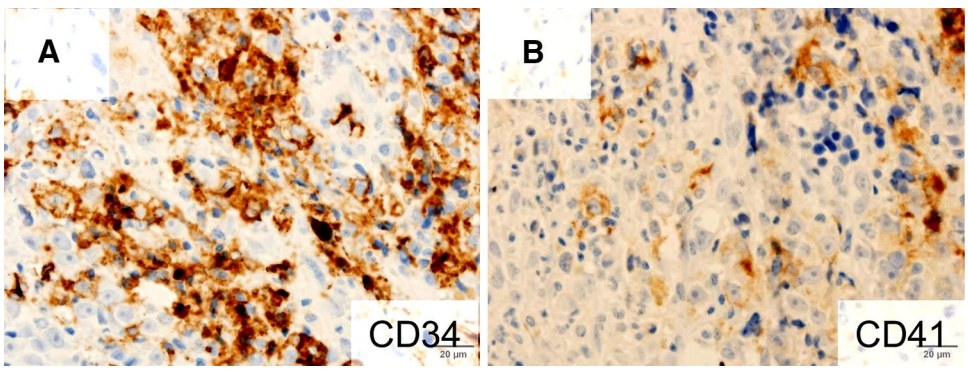

Fig. 2 Histopathological findings of the tumor. Histopathological investigation of the biopsied tumor from the left humerus revealed infiltration of monotonous blastic cells that expressed CD34 (**a**) and CD41 (**b**)

that their interaction avidity, which depends on their allelic polymorphisms, determines the magnitude of NK cell immunity [15, 23]. The association between KIR3DL1 and HLA-B on NK cell immunity and interaction avidity is worth considering in this case study. Giebel reported that the frequencies of KIR3DL1$^+$ NK cells were 0–42% with the median of 17.1% at days 28 and 56 [24]. Compared with this, recovery of KIR3DL1$^+$ NK cells in our case was quick and their frequencies were higher (41.7% and 86.0% at days 28 and 41, respectively). It suggests that quick recovery of KIR3DL1$^+$ NK cells and physical interaction of KIR3DL1 and HLA-B might suppress antitumor NK cell immunity and result in CML relapse. We obtained agreement to report as a case report from bereaved family, but no consent of the additional examination. Therefore, allelic genotyping of the donor *KIR3DL1* was not performed, and we cannot confirm that the KIR3DL1$^+$ NK cells derive from the donor. However, as we confirmed that the patient achieved complete chimera status, we think that they were donor NK cells. In addition, evaluation of KIR3DL1 protein expression by flow cytometry enabled to predict the donor KIR3DL1 allele [20]. As some NK cells had a KIR3DL1^{low} phenotype after transplant (Fig. 1d), we speculated that the donor had the *KIR3DL1*005* or **007* allele [15, 16, 25]. It is then assumed that the combination of donor KIR3DL1 and recipient HLA-B is an "interacting" pair (KIR3DL1^{low}/HLA-Bw4-80I) [17]. Collectively, physical interaction between KIR3DL1 expressed by reconstituted donor-derived NK cells and recipient HLA-Bw4 could down-regulate NK cell immunity against CML after transplant.

While our patient exhibited aggressive clinical manifestations of megakaryoblastic transformed CML with extramedullary disease consistent with those described in a previous case report [26], the etiology and clinical features of this disease subtype remain uncertain due to its rarity [26, 27]. Megakaryoblastic leukemia cells generally produce TGF-β [28], which can inhibit NK cell differentiation and induce insubstantial activation, leading to tumor progression [29]; megakaryoblastic transformed CML could, therefore, escape from NK cell immune surveillance, which is consistent with an aggressive clinical course.

We present sequential analyses of *BCR–ABL* levels and NK cell frequency and activity in a patient given *HLA*-matched, *KIR3DL1* allelic-mismatched allo-HSCT for CML in blastic crisis. Our findings indicate that consideration as for specific combinations of allelic polymorphisms of *KIRs* and *HLAs* when selecting the donor may provide improved outcome in CML patients after allo-SCT, though further investigation is now required to confirm.

Compliance with ethical standards

Conflict of interest Shinya Kimura received research grants from Bristol-Myers Squibb, Novartis, Pfizer, and Otsuka Pharmaceutical. The authors have no potential conflicts of interest to declare.

Informed consent Informed consent was obtained from the patient for publication of this case report.

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